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Genetic characterization of viable *Toxoplasma gondii* isolates from stray dogs from Giza, Egypt

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ABSTRACT

Stray dogs are considered as sentinels in the epidemiology of *Toxoplasma gondii* because they are carnivores and eat variety of foods, including garbage. In the present study, tissues and sera of 51 stray dogs (*Canis familiaris*) from Giza, Egypt were examined for *T. gondii* infection. Sera were examined for antibodies to *T. gondii* by the modified agglutination test (MAT); 50 of 51 (98%) were seropositive with titers of 20 in four, 40 in four, 80 in one, 100 in eight, 200 in 17, 400 in 11, 800 or higher in five. Hearts of 43 seropositive dogs were bioassayed in mice. Viable *T. gondii* was isolated from 22 dogs; these isolates were designated TgDogEg1 to TgDogEg22. DNA isolated from cell culture derived tachyzoites of 22 isolates was genotyped using 10 PCR-restriction fragment length polymorphism markers (SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico). The results revealed three genotypes and one mixed infection. The three genotypes are ToxoDB PCR-RFLP #2 (type III, four isolates), #3 (type II variant, 11 isolates), #20 (six isolates), 1 mixed infection. These results revealed the dominance of clonal type II, III and ToxoDB #20 lineages of *T. gondii* in stray dogs from Giza, Egypt.

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1. Introduction

The protozoan parasite *Toxoplasma gondii* infects virtually all warm-blooded animals, including birds, humans, livestock, and marine mammals (Dubey, 2010). Humans become infected post-natally by ingesting tissue cysts from undercooked meat, or by consuming food or drink contaminated with oocysts. However, only a small percentage of exposed adult humans or other animals develop clinical signs of disease. It is unknown whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or to other factors. Recently, attention has been focused on the genetic variability among *T. gondii* isolates from apparently healthy and sick hosts (Grigg and Sundar, 2009). Severe cases of toxoplasmosis have been reported in immunocompetent patients in association with atypical *T. gondii* genotypes (Ajzenberg et al., 2004; Demar et al., 2007, 2011; Elbez-Rubinstein et al., 2009; Vaudaux et al., 2010).

Historically, *T. gondii* was considered to be clonal with low genetic diversity and grouped into three types I, II, III (Dardé et al., 1992; Howe and Sibley, 1995; Ajzenberg et al., 2002a,b, 2004; Dubey, 2010). However, recent studies have revealed a greater genetic diversity of *T. gondii*, particularly isolates from domestic animals in Brazil and wildlife in USA (Dubey et al., 2011a,b; Khan et al., 2011; Su et al., 2012). Overall, information concerning isolates from Africa and Asia is scanty. We have initiated studies

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on genetic typing of *T. gondii* isolates from Egypt and other African countries (Dubey et al., 2003; Lehmann et al., 2006; Velmurugan et al., 2008; Al-Kappany et al., 2010b). Here, we report genotyping of isolates from dogs from Egypt.

Stray dogs are considered sentinels for *T. gondii* infection because they are carnivores, and eat variety of foods including garbage. Although dogs do not shed *T. gondii* oocysts but, they have been associated as a potential risk factor for *T. gondii* infection in humans due to mechanical transmission of oocysts (Frenkel and Parker, 1996; Lindsay et al., 1997). It has been hypothesized that dogs roll in cat feces and thus their hair become contaminated with oocysts. Humans might acquire *T. gondii* infection by petting dogs that have rolled over in infected cat feces, and viable *T. gondii* oocysts have been found in dog feces (Schares et al., 2005). The proportion of dogs that become infected by ingesting oocysts, tissue cysts, or congenitally is not known. There is no recent survey of *T. gondii* infection in dogs in Egypt. Two old report indicated the presence of *T. gondii* antibodies in dogs from Cairo. Khaled et al. (1982) found dye test antibodies in 28% of 43 dogs and Rifaat et al. (1977) reported dye test antibodies in 46.5% of 82 dogs. Here, we report prevalence of *T. gondii* from dogs from Giza, Egypt, and molecularly characterize *T. gondii* isolated from these stray dogs.

2. Materials and methods

2.1. Stray dogs

A total of 51 stray dogs was supplied by a contractor from Abou-rawash, Giza, Egypt (Latitude 30°1′0″N, Longitude 31°13′0″E). The climate of Giza is of semi-Mediterranean/semi-continental, but often with high humidity due to the Nile River valley's effects. In summer, the highest temperatures rarely surpass 40 °C and lows drop to about 20 °C, while temperatures in winter range from 12 °C to 17 °C. Rainfall is sparse, but sudden showers do cause flooding.

Dogs were captured and supplied to the Parasitology Department, Faculty of Veterinary Medicine, Cairo University, Egypt then they humanely euthanized and blood samples were collected from heart with the information such as sex, age; there were 20 females, 31 males, and six dogs were younger than 1 year, and 45 were older than 1 year. Also from each dog a part of heart was obtained for bioassay. Blood was allowed to clot at room temperature, and then centrifuged at 1800 × *g* for 10 min. Sera were separated and stored at −20 °C until transportation. Samples were transported refrigerated by air from Egypt to the Animal Parasitic Diseases Laboratory, United States Department of Agriculture, Beltsville, MD, where all testing was done.

2.2. Detection of T. gondii antibodies in dog serum

Sera from dogs were first screened for *T. gondii* antibodies at 1:25, 1:100, and 1:500 serum dilution with the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987). Subsequently, sera were retested starting at 1:5–1:3200 dilutions.

2.3. Isolation of viable T. gondii from dog tissues by bioassay in mice

Hearts of 43 seropositive (MAT, ≥25) dogs were bioassayed for *T. gondii*. The antibody titers of eight dogs not bioassayed were: 20 or lower in five, 40 in one, and 200 in two. Myocardium (30 g) from each dog was digested in pepsin and bioassayed in mice as described (Dubey, 2010). Briefly, tissues were homogenized in saline (0.85% NaCl), mixed with acidic pepsin, and incubated in a shaker water bath for 60 min at 37 °C. The homogenate was filtered through two layers of gauze, centrifuged, sediment neutralized with sodium bicarbonate, centrifuged again, mixed with antibiotics, and the homogenate inoculated subcutaneously into two Swiss Webster (SW) outbred albino mice, and two gamma interferon gene knockout (KO) mice (Dubey, 2010). All inoculated mice were observed daily for illness. Dead mice, or killed when ill, were examined for *T. gondii* by making impression smears from the lung and examined for tachyzoites.

Survivors were bled on day 41 post-inoculation (p.i.) and a 1:25 or 1:50 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 47 or 48 days p.i. and brains of all mice were examined for tissue cysts as a squash preparation as described (Dubey, 2010). If tissue cyst were not found in brain smears of seropositive mice, homogenized brain was sub-passaged into two KO mice.

2.4. In vitro cultivation

T. gondii isolates from positive dogs were cryopreserved in liquid nitrogen using homogenates of tissues from infected KO mice. Also the isolates cultivated in CV1 cells and tachyzoites resulted from cell cultures were cryopreserved in liquid nitrogen for future studies.

2.5. Genetic characterization

T. gondii DNA was extracted from cell-cultured tachyzoites and strain typing was performed using the genetic markers SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico as described previously (Su et al., 2010).

2.6. Ethics

All investigations reported here were approved by the institutional animal use protocol committee of the United States Department of Agriculture and the Cairo University, Egypt.

3. Results

Antibodies to *T. gondii* were found in 50 of 51 dogs with titers of 20 in four, 40 in four, 80 in one, 100 in eight, 200 in 17, 400 in 11, 800 or higher in five. One dog had no detectable antibodies at 1:5 dilution. Antibodies were found in 20 of 20 females, and 30 of 31 males, in six of six young (<1 year old), and 44 of 45 dogs older than 1 year.